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DATA EVALUATION RECORD
§ 72-3 - ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE FISH

1. **CHEMICAL:** Imazamox PC Code No.: 129171

2. **TEST MATERIAL:** AC 299263 Purity: 97.1%

3. **CITATION:**

Authors: C.E. Olivieri, G.W. Christensen, J.P Magazu
Title: Acute Toxicity of AC 299263 (Imazamox) Technical to the Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Test Conditions
Study Completion Date: March 24, 1998
Laboratory: T.R. Wilbury Laboratories, Inc., Marblehead, MA
Sponsor: American Cyanamid Company, Agricultural Products Research Division, Princeton, NJ
Laboratory Report ID: ECO 97-218
MRID No.: 445652-01
DP Barcode: D246736

4. **REVIEWED BY:** Karl Bullock, M.S., Environmental Scientist, Golder Associates Inc.

Signature: Karl Bullock **Date:** 10/19/99

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

Signature: P. Kosalwat **Date:** 10/19/99

5. **APPROVED BY:**

Signature: [signature] **Date:** 7/25/2000 8/2/00

6. **STUDY PARAMETERS:**

Age or Size of Test Organism: Mean weight: 0.28 g
Definitive Test Duration: 96 hours
Study Method: Flow-Through
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using an estuarine fish. Based on mean measured concentrations, the 96-hour LC_{50} was determined to be >94.2 ppm ai, and AC 299263 will be classified as practically non-toxic to the sheepshead minnow. The NOEC was determined to be 94.2 ppm ai.

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Results Synopsis

LC_{50}: >94.2 ppm ai 95% C.I.: N/A

NOEC: 94.2 ppm ai Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: See comments in Section 14.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. Fish lengths and the range of fish weights were not reported.

2. The deviation in pH (1.3 units) was greater than recommended by guidelines (≥0.8 units).

3. Although the range-finding test did not show any effects on the test organisms at concentrations up to 100 ppm, the test material was tested only up to concentrations of 97 ppm ai.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the sheepshead minnow (*Cyprinodon variegatus*) or the silverside (*Menidia* spp.).	*Cyprinodon variegatus*
Mean Weight 0.1-5 g	Mean: 0.28 g Range: Not reported
Mean Standard Length Longest not > 2x shortest	Mean: Not reported Range: Not reported
Supplier	Aquatic BioSystems, Inc., Fort Collins, CO

Guideline Criteria	Reported Information
All fish from same source?	Yes
All fish from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	Not fed 48 hours prior to or during testing.
Pretest Mortality < 3% mortality 48 hours prior to testing	<3% mortality in the 48 hours prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Reconstituted seawater or seawater from a natural source.	Natural seawater, which was carbon filtered, adjusted to a salinity of 11 - 17‰ with deionized water and stored in polyethylene tanks where it was aerated and recirculated through particle filters, activated carbon, and an ultraviolet sterilizer.
Does water support test animals without observable signs of stress?	Yes

Guideline Criteria	Reported Information
Salinity Weekly range should not deviate by more than 6%.	15-16‰ throughout the study
Water Temperature 22°C	21.9 - 22.5°C
pH Monthly range must not deviate by more than 0.8 unit. Euryhaline: 7.7-8.0 Stenohaline: 8.0-8.3	6.8 - 8.1
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	≥86% throughout test (6.? 7.? mg/L) $\bar{X}$ = 7.4 mg/L
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	 Glass 20 L 15 L
Type of Dilution System Must provide reproducible supply of toxicant	Proportional diluter
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	5.8 vol/24 hours
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.032 g/L/day
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	None

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	A range-finding test conducted under static conditions with nominal concentrations of 6.3, 13, 25, 50, and 100 mg/L resulted in 100% survival at all tested concentrations.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Negative control and nominal concentrations of AC 299263 equal to 13, 21, 35, 58, and 97 mg ai/L.
Number of Test Organisms Minimum 10/level for static test, 20/level for flow-through, may be divided among containers	10 per replicate, 20 per treatment level
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temperature, DO, salinity, and pH were measured daily in each test chamber. Temperature was also measured continuously in one test vessel.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Yes, solutions were collected from each replicate test chamber at 0 and 96 hours and analyzed by HPLC.

12. **REPORTED RESULTS:**

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical **1. Percent of nominal** **2. Limit of detection** **3. Method validation**	1. 97 - 100% of nominal 2. 5.00 mg/L 3. Method validation recovery: 94%
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0% mortality in the control.
Raw data included?	Yes
Signs of toxicity (if any) were described?	No sublethal effects were observed.

Analytical Results

Toxicant Concentration (mg ai/L)				
Nominal	Hour of Study		Mean Measured (SD)	Percent of Nominal
	0	96		
Control	<5.00	<5.00	-	-
13	13.0	12.1	12.6 (0.57)	97
21	21.6	20.2	20.9 (0.85)	100
35	35.2	33.0	34.1 (1.30)	97
58	57.9	54.4	56.2 (2.07)	97
97	96.9	91.5	94.2 (3.12)	97

Mortality

Concentration (mg ai/L)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<5.00	20	0	0	0	0
13	12.6	20	0	0	0	0
21	20.9	20	0	0	0	0
35	34.1	20	0	0	0	0
58	56.2	20	0	0	0	0
97	94.2	20	0	0	0	0

Other Significant Results: No sublethal signs of test material toxicity were observed.

B. Statistical Results

Method: Visual observation

96-hr LC_{50}: >94.2 mg ai/L 95% C.I.: N/A

Probit Slope: N/A NOEC: 94.2 mg ai/L

13. VERIFICATION OF STATISTICAL RESULTS:

Method: Visual observation

96-hr LC_{50}: >94.2 ppm ai 95% C.I.: N/A

Probit Slope: N/A NOEC: 94.2 ppm ai

14. REVIEWER'S COMMENTS: Since the range-finding test showed no effects at concentrations up to 100 mg/L, it is unclear why the authors tested this substance only up to 97 ppm ai in the definitive test. Typically, for the test material to be classified as practically non-toxic to the test organisms, results from the test must show that the LC_{50} is $\geq$ 100 ppm ai. Results of this test show no lethal or sublethal effects at mean measured concentrations up to 94.2 ppm ai.

In the reviewer's opinion, the 96-hour LC_{50} of AC 299263 for sheepshead minnow is most likely >100 ppm ai. Therefore, this study will be accepted as **Core**, and the test material classified as practically non-toxic to the sheepshead minnow.